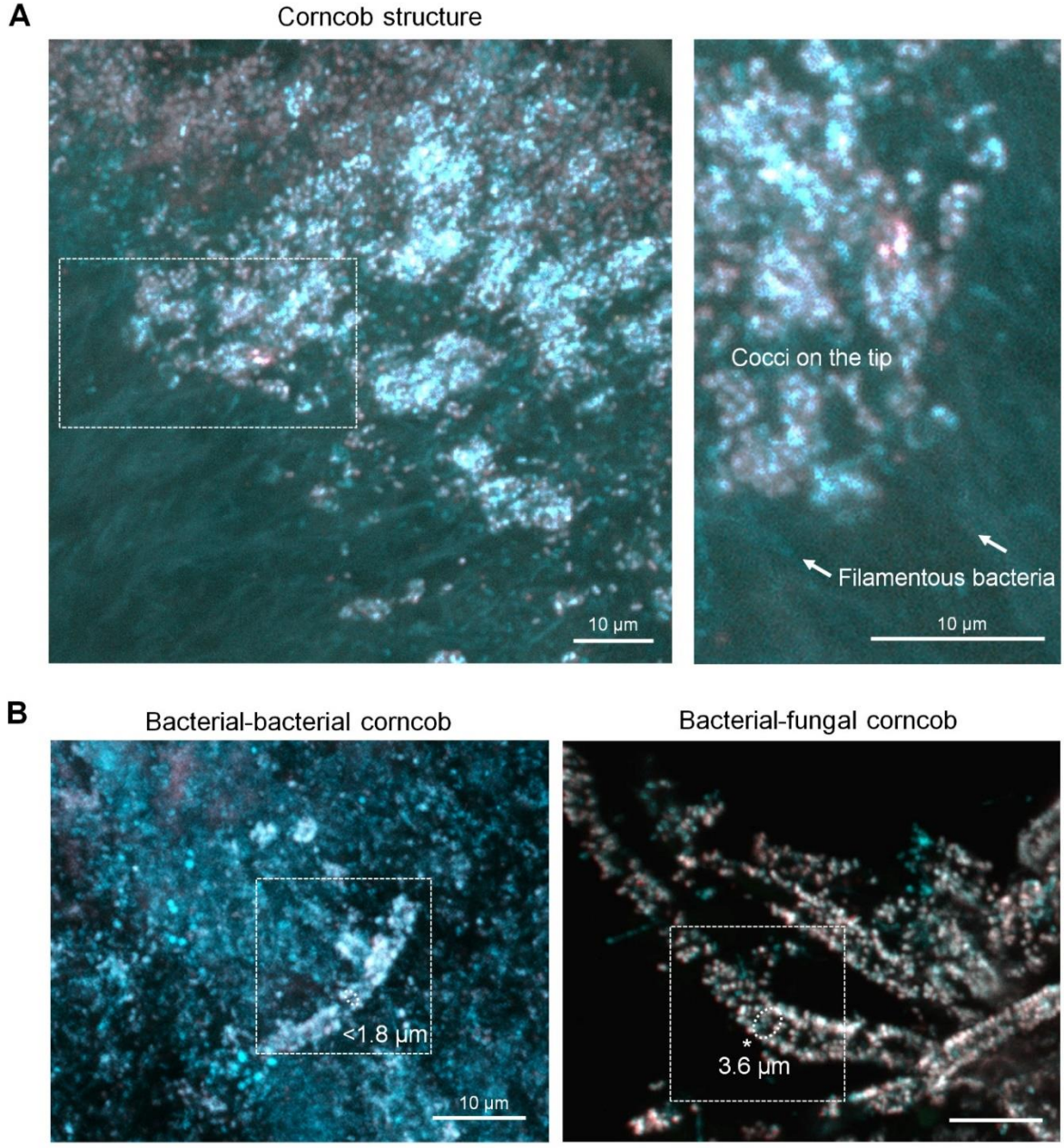
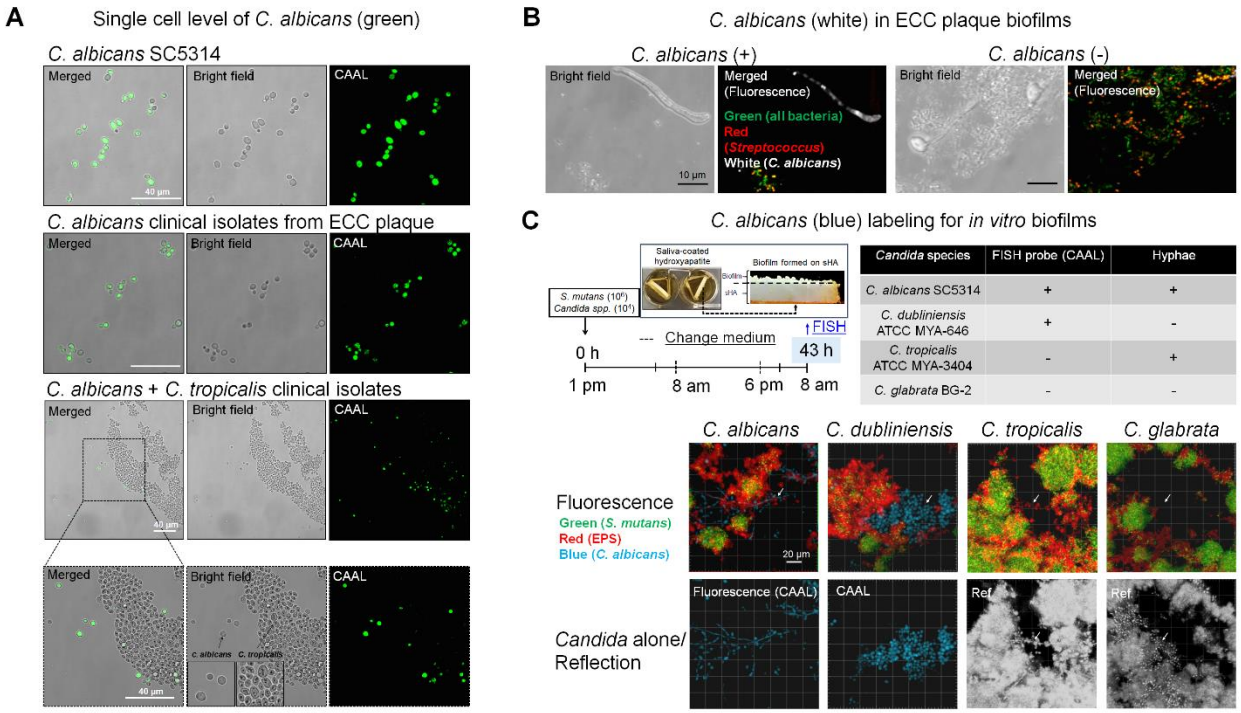
Spatial Design of Polymicrobial Oral Biofilm in Its Native Disease State

D. Kim and H. Koo

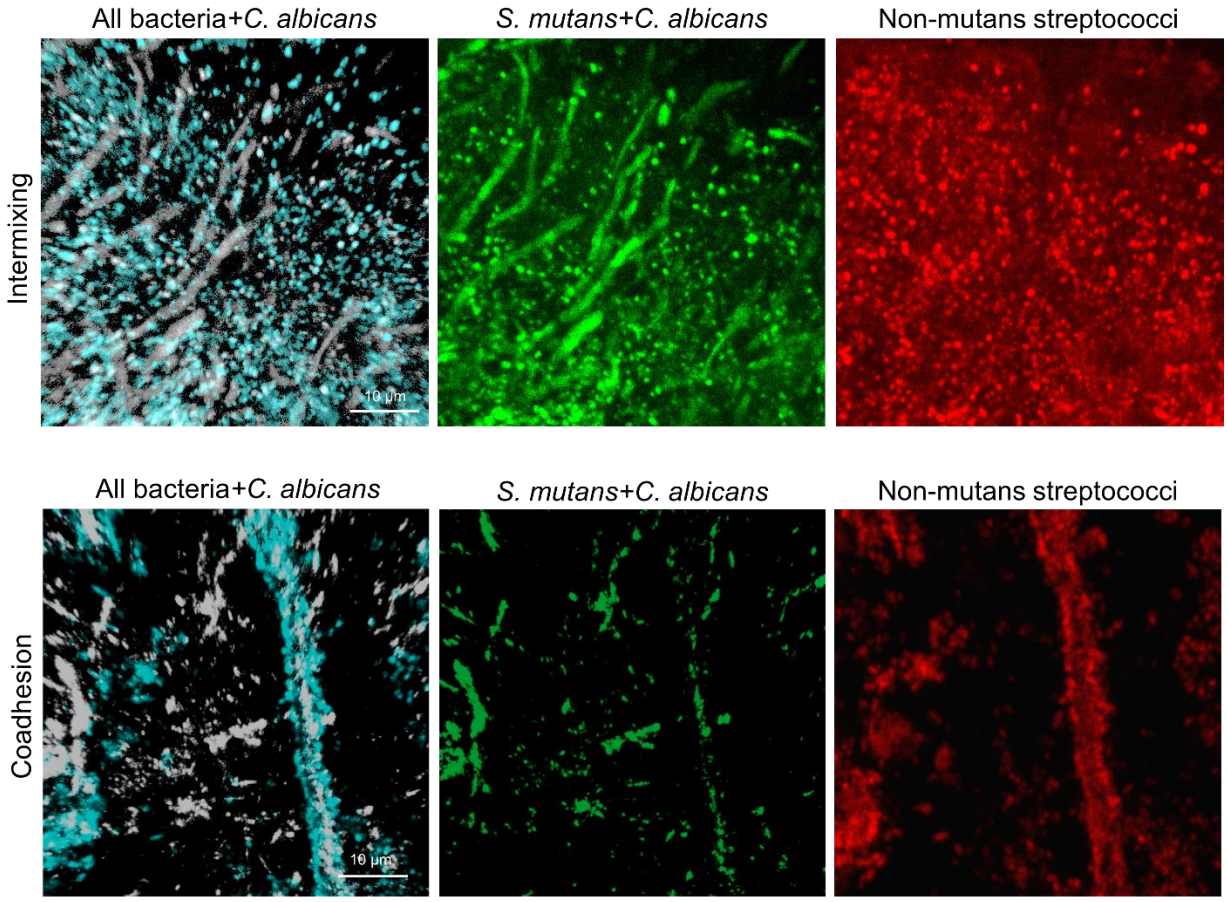
Appendix Figures



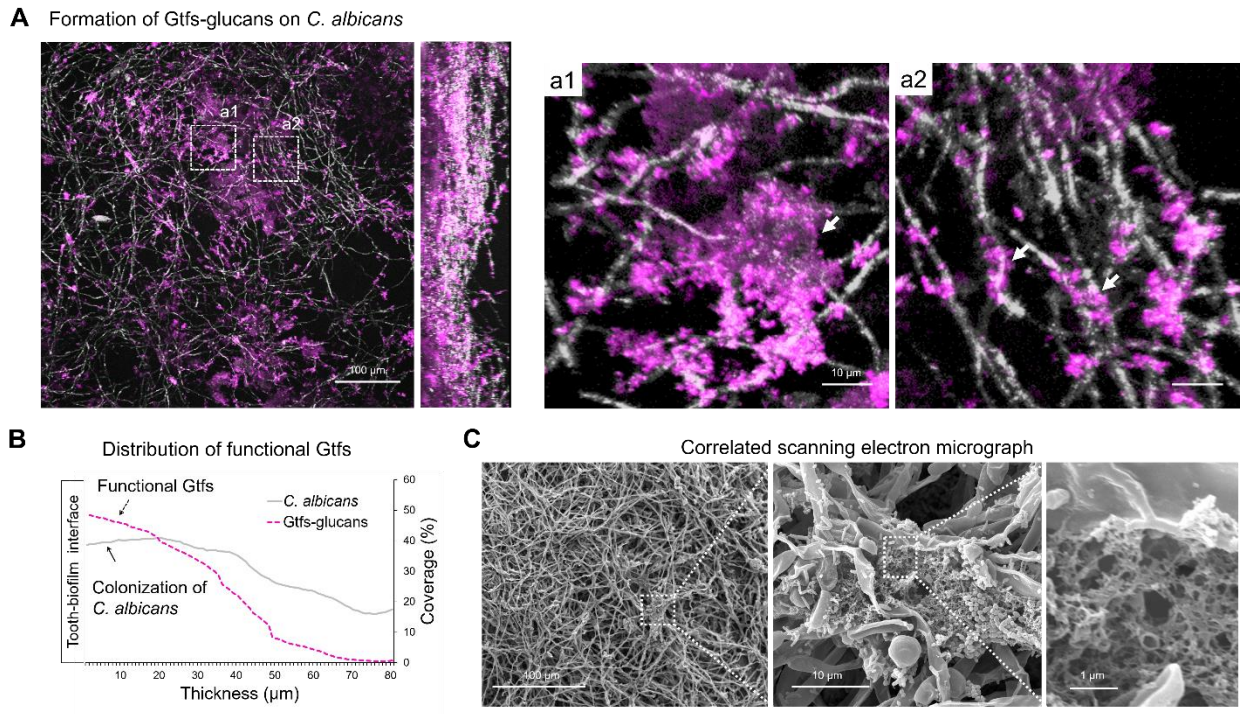
Appendix Figure 1. Streptococci in corncob structure associated with either filamentous bacteria or fungi. (**A**) Representative corncob structure on the tooth surface. (**B**) Differentiation of bacterial-bacterial or bacterial-fungal association in corncob structure via measurement of diameter of cocci surrounding filamentous cells.



Appendix Figure 2. Identification of *Candida albicans* using species-specific FISH probe and morphological difference (hyphae formation). (**A**) FISH probe validation at the single cell level of either laboratory strains or clinical isolates. *C. albicans*-targeted probe was assessed in various samples, including clinical isolate, collected plaque samples and inter-kingdom biofilm model. Briefly, clinical isolates of *C. albicans*, *C. dubliniensis* and *C. tropicalis* from the supragingival plaque collected from severe ECC children (IRB#824243), which were confirmed by colony morphology on ChromAgar (Sahand et al. 2005) and colony-PCR using species-specific primers (Donnelly et al. 1999; Baumgartner et al. 2000; Kang et al. 2008). (**B**) Application of FISH probe in the collected plaque sample from ECC children. (**C**) Differentiation of *C. albicans* and *C. dubliniensis* via FISH probe labelling and morphology assessment. To differentiate *C. albicans* in mixed-kingdom biofilm model *in vitro*, *Candida* cells including *C. albicans* SC5314, *C. dubliniensis* ATCC MYA-646, *C. tropicalis* ATCC MYA-3404, and *C. glabrata* BG-2 were grown to mid-exponential phase in ultrafiltered tryptone-yeast extract (UFTYE) with 1% (w/v) glucose at 37°C and 5% CO_2 and inoculated with approximately 10^6 (CFU/mL) of *S. mutans* and 10^4 (CFU/mL) of each of *Candida* spp. in 2.8 mL UFTYE containing 1% (w/v) sucrose at 37°C under 5% CO_2 as described previously (Falsetta et al. 2014; Kim et al. 2018). The culture medium was changed twice daily until the end of the experimental period. The extracellular polysaccharides (EPS) glucans were labelled with 1 μM Alexa Fluor 647-dextran conjugate (Molecular Probes Inc.) (Klein et al. 2009).



Appendix Figure 3. Microbial components in cross-kingdom communal organization. All bacteria (depicted as blue) merged with *C. albicans* (shown in white). Both *S. mutans* and *C. albicans* were labelled with species-specific probe with FITC-fluorophore (shown in green). Non-mutans streptococci are depicted in red.



Appendix Figure 4. Functional Gtfs activity of intact bacterial-fungal organization from diseased teeth. (**A**) Functional Gtfs activity on bacterial-fungal community across the intact biofilm. Glucans labelled with Alexa Fluor 647 on the fungal surface is indicated by arrows in a1 and a2. *C. albicans* and Gtfs-glucans are shown in white and magenta, respectively. (**B**) COMSTAT analysis of *C. albicans* and Gtfs-glucans distribution across biofilm thickness (from the biofilm-tooth interface to the uppers layers of the biofilm). The confocal images were also analyzed using COMSTAT for quantification of *C. albicans* and Gtfs-glucans. The coverage represents the overall biomass occupied by *C. albicans* with Gtfs-glucans within intact biofilms, which provide direct measurement of their amounts across the depth. (**C**) Electron micrographs of the same biofilm sample. After confocal imaging, the biofilm sample was fixed in 2% paraformaldehyde/2% glutaraldehyde overnight, and after rinsing was gradually dehydrated by ethanol (50, 70, 80, 90 and 100% for 10 min each). Sample was then dried with hexamethyldisilane and sputter-coated (Au/Pd) before imaging. The electron micrographs correlated with confocal imaging were acquired using a high-resolution scanning electron microscope (Quanta 250 FEG, FEI).

References

Baumgartner J, Watts C, Xia T. 2000. Occurrence of Candida albicans in Infections of Endodontic Origin. J Endod. 26(12):695–698.

Donnelly SM, Sullivan DJ, Shanley DB, Coleman DC. 1999. Phylogenetic analysis and rapid identification of Candida dubliniensis based on analysis of ACT1 intron and exon sequences. Microbiology. 145(8):1871–1882.

Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai C-H, Gonzalez-Begne M, Watson G, Krysan DJ, Bowen WH, et al. 2014. Symbiotic Relationship between Streptococcus mutans and Candida albicans Synergizes Virulence of Plaque Biofilms *In Vivo*. Infect Immun. 82(5):1968–1981.

Kang Y, Iida S, Yamamoto S, Kogure T, Tanaka R, Mikami Y. 2008. Trf4 is a Useful Gene for Discrimination of Candida tropicalis from other Medically Important Candida Species. Nippon Ishinkin Gakkai Zasshi. 49(1):39–43.

Kim D, Liu Y, Benhamou RI, Sanchez H, Simón-Soro Á, Li Y, Hwang G, Fridman M, Andes DR, Koo H. 2018. Bacterial-derived exopolysaccharides enhance antifungal drug tolerance in a cross-kingdom oral biofilm. ISME J. 12(6):1427–1442.

Klein MI, Duarte S, Xiao J, Mitra S, Foster TH, Koo H. 2009. Structural and molecular basis of the role of starch and sucrose in Streptococcus mutans biofilm development. Appl Environ Microbiol. 75(3):837–41.

Sahand IH, Moragues MD, Eraso E, Villar-Vidal M, Quindós G, Pontón J. 2005. Supplementation of CHROMagar Candida medium with Pal's medium for rapid identification of Candida dubliniensis. J Clin Microbiol. 43(11):5768–70.